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Pathological findings in stranded seals (Phoca vitulina and Grypus) in Noord Holland and Texel (2011-2012)

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Summary

An overview is created of the pathological findings from post mortem examination on 27 seals. 25 Of the seals were of the species Phoca Vitulina (harbor seal). In one case the species remained unknown while the head was missing. One seals was of the species Halichoerus grypus (grey seal). The seals used for this research project were either found dead on the shore or died at the animal rehabilitation centre on Texel, Noord-Holland, Ecomare. The seals varied in age, sex, date and place of stranding and whether or not euthanasia was performed.
After performing the necropsy with macroscopical evaluation, and in five cases histological research, a cause of death was determined. In five animals also histological findings were available. Twelve seals died of euthanasia (44,4%), four because of lungworm (14,8%), four seals suffered from trauma (14,8%), in four cases the cause of death was grouped as 'others' (14,8%) and in three cases the cause of death remained unknown (11,1%).
Euthanasia was always performed because of lungworms (Otostrongylus circumlitis and Parafilaroides gymnurus). With a total of 16 seals suffering from lungworm, this was determined to be the cause of death of the greatest significance. These seals were all juvenile and in a bad to moderate nutritional condition. The main macroscopical findings in the respiratory tractwere: decreased and irregular consolidation, colourful aspects, emphysema and hyperaemia and hemorrhagic fluid in the lungtissue and bronchia. No correlation between the severity of the infection, damage to the lungtissue and the amount of worms was found. Further research will be needed.
A total of 23 seals were infected with parasites anywere in the body. None of the seals used in this studie died from an infection with parasites other than the lungworm species.

Introduction

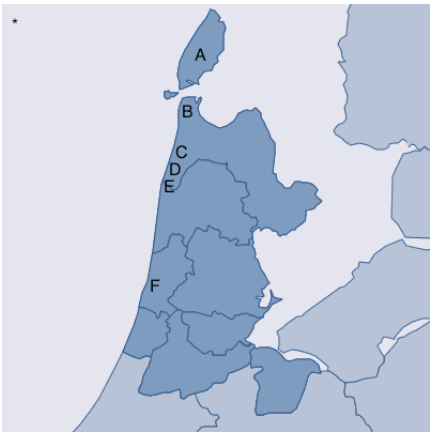
Strandings of marine mammals have occurred along the shores of the world's oceans for centuries. But the rough wetherconditions in the winter of 2011, combined with the growing population, caused rehabilitation centers in the Netherlands to become overcrowded.
A recent counting of seals in 2011, performed by Imares (Institute for Marine Resources and Ecosystem Studies) in order of the Ministery of Economics, agriculture and innovation, showed a total of 7.821 harbor seals (Phoca vitulina), including 1.445 juveniles, in the Dutch Waddensea[1]. Countings in the international Waddensea showed a total of 24.118 harbor seals. Grey seal countings in the Waddensea presented a total of 2.388 seals under which 322 juveniles.
The population growth in the Dutch Waddensea was 13% in the last two years, 5% higher than the international Waddensea. This population growth could partly clearify the great amount of strandings in 2011[2].

Harbour seals are seasonal breeders. The annual reproductive cycle of most seal species is characterized by a tight synchrony of births, ensuring that pups are born at the optimal time of year[3, 4].
The matingseason takes place between July and the beginning of August. Placental gestation starts after a period of delayed implantation[3, 5]. Once implentation has taken place, mostly between November and December, gestation takes an other eight months in the harbor seals and eight and a half in the grey seal. Pups are born in June or July. The grey and harbor seal, female reproductive cycle after parturition consists of lactation followed by oestrus and mating[4].
Lactation and weaning period takes three to six weeks. Each year during this period, or shortly after, a number of pups will get separated from their mothers. It is mainly these pups that were brought to the rehabilitationcentre Ecomare[6].

Existing literature on the strandings of marine mammals gives acces to the otherwise hard to reach species. Though consistent documentation of stranding events gives information on individual animal health it does not present the health status of the whole population[7, 8].
Investigation into the mortality trends could be usefull to estimate the health status of marine mammals globally, or in this case more specifically the Waddensea[8].
Post mortem examination of seals stranded on the Dutch coastline could give information on the currently present diseases in the seals. These insights will be of value for the Dutch institutions: Ecomare, Imares and the Dutch Wildlife Health Center.
The goal of this report is to summarize necropsy findings in Phoca vitulina and Halichoerus Grypus. And to determine the cause of death of seals that stranded at the Dutch coastline, died or were euthanised at the rehabilitationcentre Ecomare.

Material & Methods

The 27 stranded seals used for this research project were either found dead on the shore or died at the animal rehabilitation centre on Texel, Noord-Holland, Ecomare. The seals that died within 24 hours after arrival in the rehabilitation center are considered free-ranging animals.



A.	Texel,	n = 12
B.	Den Helder,	n = 4
C.	Callantsoog,	n = 2
D.	St. Maarten zee,	n = 1
E.	Petten,	n = 2
F.	Castricum,	n = 2
*	Unknown,	n = 4

Fig. 1: stranding locations, Noord-Holland

25 Of the seals were of the species Phoca Vitulina (harbor seal). In one case the species remained unknown while the head was missing. One seals was of the species Halichoerus grypus (grey seal). The seals varied in age, sex, date and place of stranding and whether or not euthanasia was performed (Table 1).

Species	*Phoca vitulina*	*Halichoerus grypus*	*Unknown*	
	25	1	1	
Sex	*Male*	*Female*	*Unknown*	
	10	17	0	
Age	*Neonate*	*Juvenile*	*Adult*	*Unknown*
	0	*20*	*7*	*0*
Stranding date	*November (2011)*	*Decemer (2011)*	*January (2012)*	*Unknown*
	3	10	12	2
Ecomare	*Yes (>24h)*	*Yes (<24h)*	*No*	*Unknown*
	11	*4*	*10*	*2*
Chip	*Yes*	*No*	*Unknown*	
	3	24	0	
Euthanasia	*Yes*	*No*	*Unknown*	
	12	14	1	

Table 1: specification of the seals, n = 27

The carcasses were stored in a -20 degrees freezer until necropsy. The seals were defrosted in a fridge a few days before necropsy. The amount of days necessary for defrostinga depends on weight and size. Necropsies were performed coording a standardized protocol (attachment nr. 1). This protocol was originally designed for necropsies of harbour porpoises and was modified for seals.
Before starting the dissection, photographs of the seals exterior and measurements were taken and a external macroscopical evaluation was performed. Photograpic documentation consisted of: the full body, the head and snout, teeth, anogenital region and external observations. Mesurements consisted of: total lenght, distance from head to tail, distance from flipper to tail and the girth of chest.
After opening the seal a nutritional condition code (NCC) and a decomposition condition code (DCC) was determined. The NCC is based on the blubber tickness, the amount of muscletissue and possible signs of recent feeding. The DCC is based on the external and internal decomposition signs of the carcass (attachment nr. 1).
A DCC 1 score was given to a very fresh, less than 48 hours dead carcass, these carcasses showed signs of rigor mortis. The DCC 2 score was given when the first signs of decomposition were visible but the eyes, skin and organs were still in a good state. When the carcasses were defrosted one to three days longer before dissectioning, the DCC 3 score was given. Skin peeling, moderate to clear signs of decomposition (change in colour and cosistency) were noticable, as well as the smell of decomposition. A DCC 4 of DCC 5 score was given to cases of which organs were clearly altered, these carcasses were not usefull for pathological examination[9]. Depending on this DCC score, different samples were taken according to the sampling list (attachment nr. 1). The higher the DCC score, the lesser amount of samples were taken or the lesser evaluations were performed. Sampling consisted of: histology, toxicology, parasitology and bacteriology.
All organs were examined macroscopically. These macroscopic evaluations were digitally documented in a macroscopy template. Affected organs were described according to the morphological diagnosis, namely: severity, time, distribution, lesion and anatomic site. Lesions were described using the terms: minor, moderate or severe. All internal abnormalities were photograpically documented.
Histological evaluation was performed on the heart, lungs, liver, pancreas, spleen, kidney, adrenal, intestine, thymus, thyroid, eye, cerebrum, cerebellum, multiple lypmhenods and on lesions or abnormalities. Tissue samples were fixed in 10% neutral buffered formaline, embedded in paraffin and 3 µm slides were routinely stained with Heamotocylin and Eosin (HE). When necessary a Zeehl-Neelsen and PAS staining were carried out. Macroscopic evaluations were performed by pathologists: J.IJzer and M. Kik. Histological evaluations were performed by J. IJzer.
When a carcass scored either a DCC one or two, samples were taken for toxicologic and bacteriologic analysis. Instruments were cleaned in water, dettol and 70% alcohol before they were used to take toxicology samples. These samples were first wrapped in aluminiumfoil and then put into a plastic bag or were put into a small cup (3x1x1 cm). Samples for bacteriological analysis were packed into plastic bags. Both toxicologic as well as bacteriologic samples were stored in a -20 degrees freezer for later use.

When present, parasites were collected during necropsies and preserved in alcohol 70% and glycerine (9:1). The intestinal parasites were collected from the contents of one meter jejunum on two third of the total intestinal length. The jejunum was cut open, the contents were rinsed out with water and collected in a plastig bucket. Contents and water were filtered with a 150µm sieve. The parasites were identified by a parasitologist (H.J. Cremers) using a binoculair. Parasitic infestation was quantified variating from mild, moderate to severe in the affected organs.
Part of the samples were taken on behalf on the Imares institute (Institute for Marine Resources and Ecosystem Studies). This set consisted of: the mandibulae for age classification, samples for toxicologic analysis and the stomach with it's contentst for determination of the food intake by skull remnants of ingested fish. These samples were all frozen and stored for later use.
At the end of each macroscopic pathological examination a possible cause of death was determined. These were grouped in the following categories: euthanasia, lungworm, trauma, others and unknown. After completion of histological evaluation a final conclusion was drawn for each animal.

Results

Overview

27 Necropsy examinations were performed on seals stranded in the winter of 2011-2012. The seals variated in age (20 juvenile, 7 adults) and sex (17 female, 10 male). In table two, data of all the individuals are summarized.

Animal	Date	Age	Sex	DCC	NCC	-20℃	BW	Ecomare	Macro conclusion
HG 14	19-01-2012	J	F	2	4,5	Y	14		Trauma or hypothermia
PV 79	12-12-2011	J	M	2	5	Y	16	2 days	Euthanasia in rehab
PV 80	24-12-2011	J	F	5	-	Y	13		Unknown
PV 81	18-11-2011	A	F	2	3,4	Y	53		Unknown
PV 82	16-11-2011	A	F	3	1	Y	56		Trauma
PV/HG 83	28-11-2011	J	M	3	3	Y	15		Chronic pneumonia
PV 84	12-12-2011	J	F	2	3	Y	17	3 days	Euthanasia in rehab
PV 85	17-12-2011	A	M	2	2	Y	71,5		Torsio of the jejunum trough an opening in the mesenterium
PV 86	21-12-2011	A	F	3	2	Y	75,5		Unknown
PV 87	17-12-2011	J	F	2	5	Y	17		Parasitic infestation and damage to the lungtissue
PV 88	26-12-2011	J	M	2	3	Y	18	13 days	Pneumonia, lungworm
PV 89	5-12-2011	J	F	2	3	Y	13	23 days	Euthanasia in rehab
PV 90	17-12-2011	J	M	2	3	Y	17	12 days	Euthanasia in rehab
PV 91	27-12-2011	J	F	2	3	Y	17	13 days	Euthanasia in rehab
PV 92	6-2-2012	A	F	3	1	Y	81		Blunt trauma
PV 93	-	J	F	2,3	4	Y	15	Unknown	Euthanasia in rehab
PV 94	20-1-2012	A	M	3	4	Y	62		Sepsis because of old wounds?
PV 95	18-1-2012	J	M	3	3	Y	19		Pneumonia, lungworm
PV 96	19-1-2012	J	F	3	3	Y	37,4		Blunt Trauma
PV 97	18-12-2011	J	M	2	3	Y	16	28 days	Euthanasia in rehab
PV 98	18-1-2012	J	F	2	4	Y	14	2 days	Euthanasia in rehab
PV 99	19-1-2012	J	F	2	4,5	Y	14	1 day	Euthanasia in rehab
PV 100	20-1-2012	J	F	2	3,4	Y	19,5		Extensive necropurulent peritracheitis
PV 101	14-1-2012	J	M	1	3	N	12,5	17 days	Euthanasia in rehab
PV 102	18-1-2012	J	M	1	2	N	19	13 days	Euthanasia in rehab
PV 103	30-1-2012	J	F	1	3	N	18	1 day	Euthanasia in rehab
PV 104	30-1-2012	J	F	1	3	N	19,5	1 day	Purulent inflammation in multiple organs

Table 2: overview of the seals, n = 17

HG: Halichoerus grypus
PV: Phoca vitulina
Date: date of stranding, day – month - year
Age: J = juvenile, A = adult
Sex: M = male, F = female
DCC: Decomposition code
NCC: Nutritional condition code
-20℃: Y - the carcas has been frozen, N - the carcas was fresh
BW: Bodyweight in kilograms
Ecomare: Number of days in rehabilitation in Ecomare

From five animals, histological evaluation was completed within the time frame of this three month project. Results are summarised in table 3.

Species	Nr.	Microscopical conclusion
PV	79	*Lnn mesenterialis:* Splendore-Hoeppli fenomenen (eosinophilic, radiar depositions in paren-chyma)/ Small necrotic foci around parasitic fragments, surrounded by eosinophilic debris *Lnn bronchialis:* Active lymphfollicels in the cortex *Spleen:* Lymphoid activity in white pulpa/ Minor extramedullary heamopoesis in red pulpa *Lungs:* Less air containing parts/ Hyperplasia of the bronchial epithelium
PV	81	*Lnn mesenterialis:* Splendore-Hoeppli fenomenen (eosinofilic, radiar depositions in paren-chyma)/ Small necrotic foci around parasitic fragments, surrounded by eosinophilic debris
PV/HG	83	*Lnn mesenterialis:* Splendore-Hoeppli phenomenon (eosinofilic, radiar depositions in the parenchyma) *Lungs:* Multiple crosswise cut nematoda, surrounded by cellulair infiltrates
PV	86	*Spleen:* Lymphoid activity in the white pulpa/ Minor extramedullary heamopoesis in the red pulpa. *Liver:* Small focus with lytical necrosis, and necrotic hepatocytes, cellular debris. *Lnn Prescapularis:* Signs of recent bleeding in surrounding fat tissue, erythrocytes in sinusses.
PV	88	*Liver:* Hyperaemia/ Bileduct proliferation

Table 3: overview of the macroscopical conclusions, n = 5
HG: Halichoerus grypus
PV: Phoca vitulina

Decomposition and nutritional scores

An overview of the given DCC and NCC scores is presented in the figures two and three. Most animals (55,6 %) recieved a DCC 2 status, these carcasses were frozen and defrosted but still usefull for pathological examination.
The NCC score is based on the blubber thicknes, stomach contents and weight. Most carcasses were scored a NCC 3, these animals were not yet emaciated but very thin and in a bad nutritional condition.

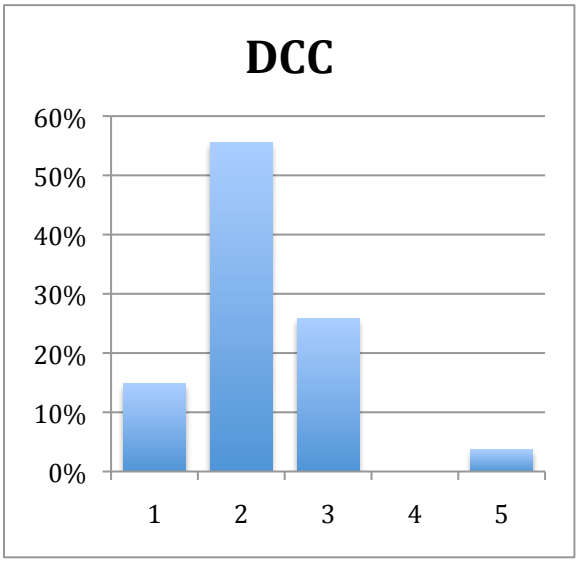


Fig. 2: DCC scores, n = 27

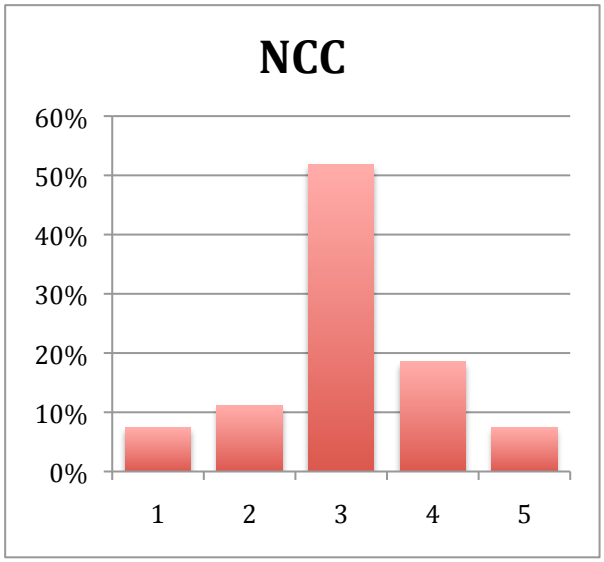


Fig. 3: NCC scores, n = 27

The probable cause of death was determined in 89% of the cases (24/27). The cause of death remained unknown in three cases. In five animals (Table 3) also histological evaluation could be included in the conclusion. The main findings are presented in tabel 4.

	Probable cause of death after macroscopical evaluation					
Pathological findings	Euthanasia	Lungworm	Trauma	Others	Unknown	Total
	n = 12 44,4 % %	n = 4 14,8 %	n = 4 14,8 %	n = 4 14,8 %	n = 3 11,1 %	n = 27

Table 4: probable cause of death determined after macroscopic examination

Euthanasia

44,4 % (N = 12) of the stranded animals were euthanised at Ecomare, or in one case, at the beach. All animals (100%) were euthanised because of, a suspected to be severe, lungworm infection. 25% (n=3) of these animals were dissected while fresh and have never been frozen.
83% (N = 10) of the euthanised animals were first given treatment before euthanisation. The treatment ranged between two and twenty-eight days, with an avarage of twelve days. Two seals where euthanised directly at arrivel at Ecomare because of their very poor condition. The animals relapsed after an intensive treatment with antibiotics (tetracylins), anthelmintics (ivomectin) and corticosteroids (dexamethason). Due to this treatment, it was not possible to form an objective diagnosis on the initial severity of the parasitic infection.

Lungworm infections

Besides the animals that were euthanised, four other seals died from an infection with lungworm. These seals were all juvenile and in a bad to moderate nutritive condition.
As in the euthanised animals, the damage to the lungtissue was not consistend with the severity of the parasitic infection. A minor infection could give major damage to the lungtissue while a major infection only caused moderate damage. It was possible though to rate the amount of damage (mild, moderate, severe) caused by the parasites.

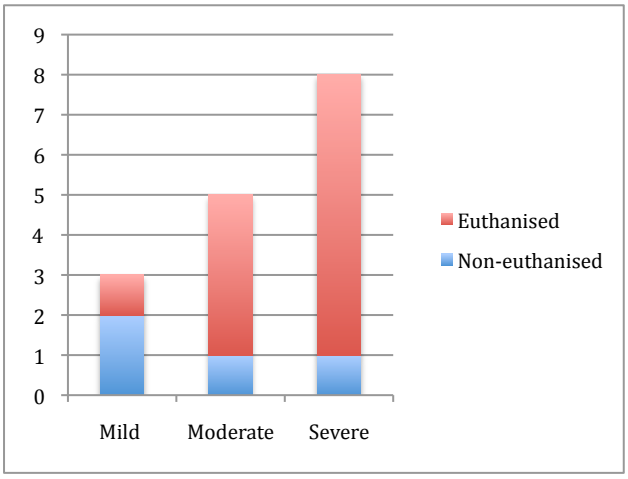


Figure 4: parasitic infestation of the lungs in the euthanised seals, n = 16

At necropsy, parasites were mainly found in the caudal and ventral lobes of the lungs.
The main pathological findings in the lungs were:

- Decreased consolidation of the lungtissue
- Irregular consolidation
- Colourfull aspects
- Emphysema (possibly postmortem)
- Hyperaemia and hemorrhagic fluid in the lungtissue and bronchia

Trauma

Four animals (14,8%) died because of blunt trauma. All these animals showed haemorrhages in the blubber, and when more severe also in the muscles or retroperitoneal. These haemorraghes were mostely located around the shoulderblades but also on different places like the tail, ribcage, trachea, head and neck.
In one out of four animals, the hemorraghes were much more extensive. This animal had a broken spine and bleeding in the different tissue layers. In the other carcasses the heamorrhages were less severe and located only in the blubber or superficial musclegroups. None of these animals were rehabilitated at Ecomare wich excludes forced feeding or euthanasia as a cause for the heamorrhages around the shoulderblades.

Others

Besides the previously mentioned causes of death a few other findings should be described. Four different pathological findings could not be grouped in the earlier determined categories. The etiology remained unknown.
One of the adult males showed a torsio of the jejunum, trough an opening in the mesenterium. This male was in a good nutritive condition. An other adult male, also in a good nutritive condition had old wounds, which were already healing. This animal could have died from a sepsis, caused by the old wounds.
Two juvenile females suffered from purulent inflammation. One of them had this purulent inflammation in multiple organs. The other female showed an extensive necropurulente peritracheitis. In both cases the cause remained unknown.
33% of the carcases showed bleeding on different places. These heamorrhages were not significant enough to be categorized as the cause of death. The heamorrhages were mainly located on the ribcage, behind the shoulder and between the mandibulae. Five out of ten animals that showed bleeding around the shoulderblades were treated in Ecomare. In these cases the heamorrhages might be caused by forced feeding, administering of medication or euthanasia. In these shelter seals, these limited hemorrhages were not considered as the cause of death.

Histological results (n = 5)

The histological samples were difficult to judge due to the freezing artefacts and autolysis. Whitin this range, abnormalities were found in the spleen, lungs, liver and in different lymphnods. The main observations were:

- *Spleen:*

- Lymphoid activity in the white pulpa
- Minor extramedullary heamopoesis in the red pulpa

- *Lungs:*

- Multiple cross-sections of nematoda, surrounded by cellulair infiltrates
- Less air containing parts
- Hyperplasia of the bronchial epithelium

- *Liver:*

- Small focus with lytical necrosis, and necrotic hepatocytes, cellular debris.
- Hyperaemia
- Bileduct proliferation

- *Lymphnodes:*

- *Lymphonodulus mesenterialis:*
    - Splendore-Hoeppli fenomenen (eosinofilic, radiar depositions in the parenchyma)
    - Small necrotic foci around parasitic fragments and eggs of ascocotyle septentrionalis, surrounded by eosinophilic debris
- *Lymfnodulus bronchialis:*
    - Active lymphfollicels in the cortex
    - Fibrine depositions, possible necrosis

Parasitology

All animals were examined on the presence of parasites. 15 Out of 27 animals were tested positive for parasites in the lungs, 11 for parasites in the intestine and 20 for parasites in the stomach. Results are displayed in graphics five to seven.

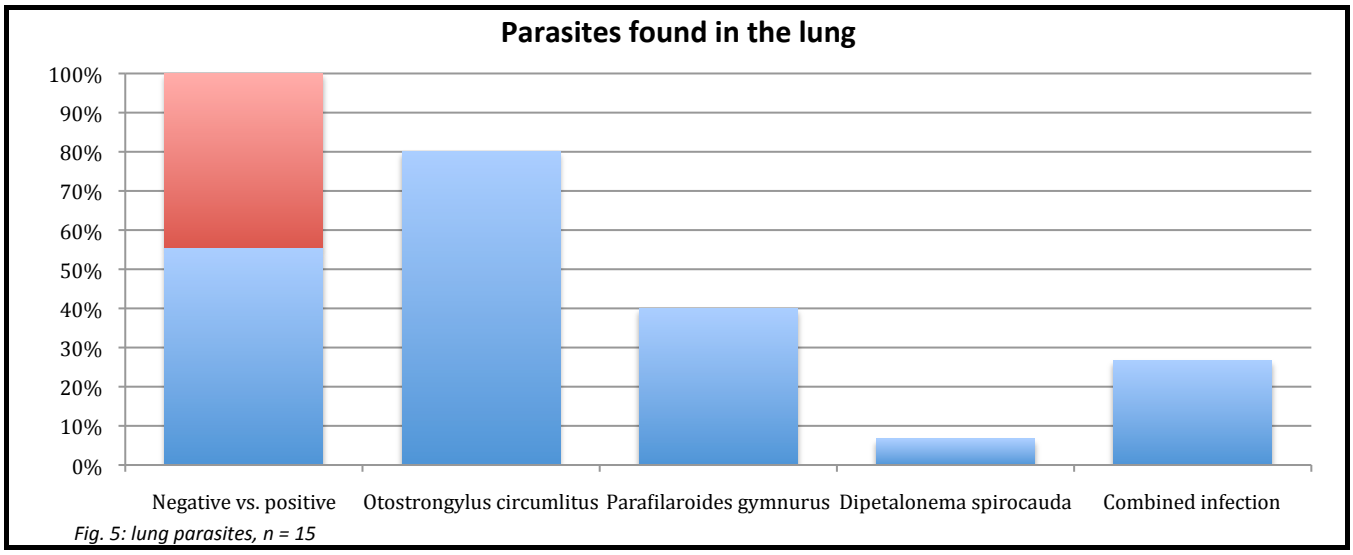


Fig. 5: lung parasites, n = 15

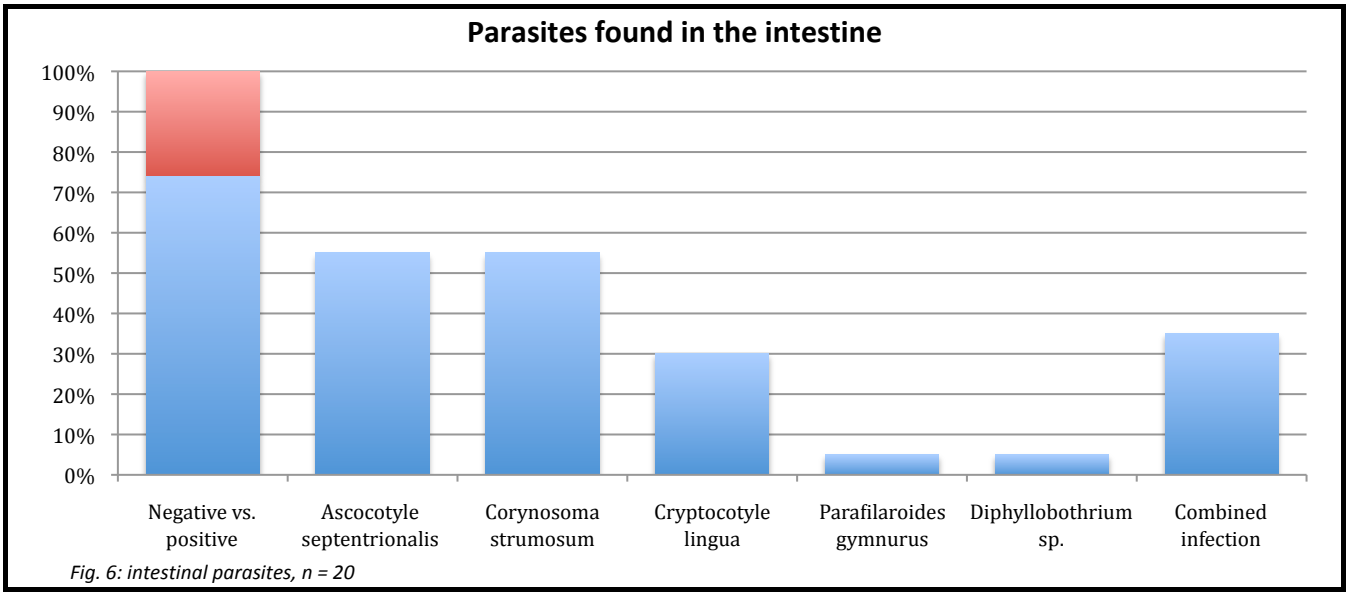


Fig. 6: intestinal parasites, n = 20

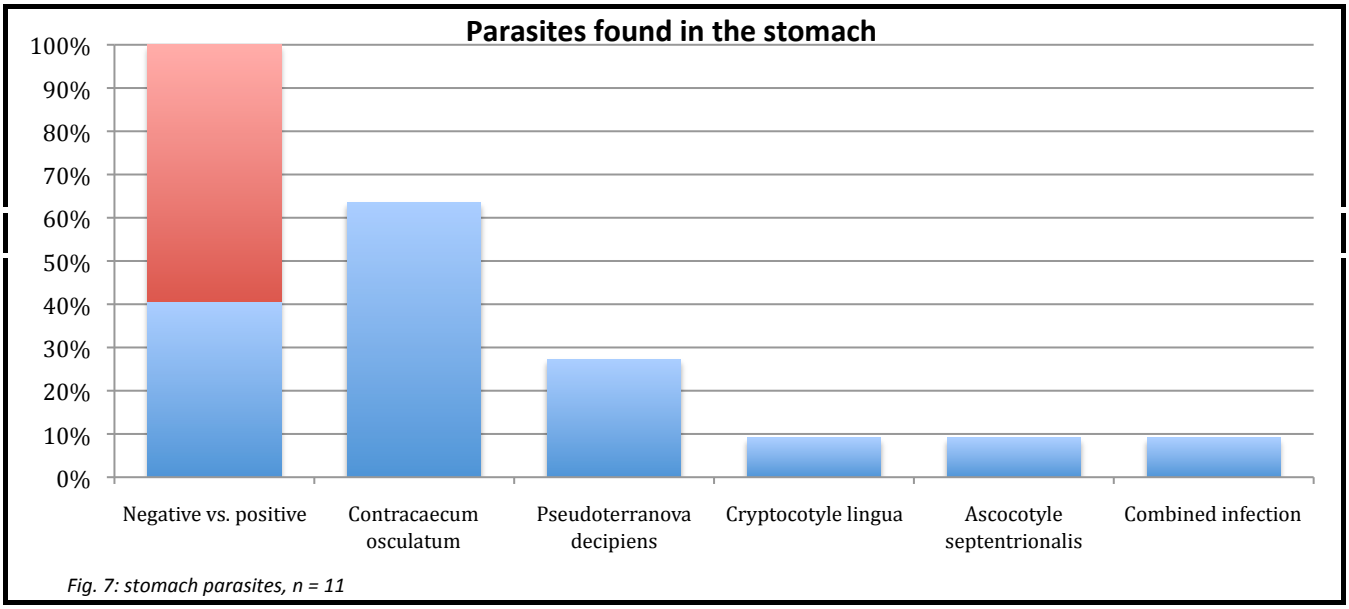


Fig. 7: stomach parasites, n = 11

Discussion

Results

The number of necropsy examinations performed in this study (n = 27) was lower than in similar studies. In the limited time available, 23 necropsies were scheduled in advance. Because of great help a total of 27 necropsies could be completed. Other evaluations could not be performed because of the strict timeframe. Histological evaluation was available on 19% of the animals instead of on all suitable animals. This was caused by underestimating the time needed to complete the preparation of the histology samples. Histological evaluation of the completed samples was limited by freezing artefacts and autolysis.
The animals variated in age, sex and stranding location. 20 Out of 27 animals were juvenile, compaired to only 7 adult animals. The male-female ratio was 10-17. The majority of the animals (n = 12) stranded on Texel, the remaining 15 animals stranded along the coast of Noord-Holland.
The 27 completed necropsies resulted in a macroscopical conclusion on the cause of death in 89% of the cases. Lungworms being the most significant (16 out of 27 animals). This conclusion though, is not representive for the whole seal population of the Dutch Waddensea. Whilst the animals in this studie were not randomly picked.
56 % of all the animals stayed in Ecomare for a few hours to multiple days. Twelve of these animals have been euthanised because of lungworm. In the wild population a greater variety in the causes of death was found. This would also point out that the animals used in this project are not representive for the population of the Dutch Waddensea. Nevertheless a clear overview of the pathological findings was created.

Parasites

Other studies have shown that the harbour seal, as a fish-eating animal at the end of the food-chain, is parasitized by many worm species from different groups[10].
The results in this study confirm the presence of a wide variety of parasites. There are two species in particulair that were of greater significance. These are the lungworm species: Otostrongylus circumlitus (Nematoda, Strongylida, Crenosomatidae) and Parafilaroides gymnurus (Nematoda, Strongylida, Filaroididae). These parasites were found in 52% of all the examined animals. And were defined as the cause of death in 16 cases.
O. Circumlitis, the large lungworm, resides mainly in the trachea, bronchi and large bronchioles, and causes obstructions which are associated with catarrhal or purulent bronchitis and bronchopneumonia. P. Gymnurus, a small lungworm, resides in the alveoli and bronchioles and is associated with bronchioloitis and bronchopneumonia, with pulmunary atelactasis and emphysema[11-13].
Whilst infections with Parafilaroides species are generally considered more pathogenic than infections with O. Circumlitis[14]. The results in this studie have not shown this correlation, mainly because of the amount of combined infections.
There was also no correlation found between the severity of the infection and the amount of macroscopical damage to the lungtissue. When there would have been a linkage between these factors it would not have been objective because of the treatment that some animals recieved at Ecomare.
The pathogenicity of other helminth species is regarded as low. Intestinal trematodes and the acanthocephala even in high numbers do not cause clinical signs, however, they can cause local inflammation and ulcers in the gut with a higher risk on secundairy bacterial

infections[15]. Non of the seals used in this studie have died from an infection with parasites other than the lungworm species.
Parasites were also found in the histological samples. Microscopical research performed on these samples showed eggs of the Ascocotyle septentrionalis in the mesenterical lymphnodes. This could prove that this parasite is able to migrate outside of the intestinal tract.

Ecomare

15 Animals in this study were taken in to the rehabilitation centre Ecomare. These animals died, despite the dedication of the animal caretakers. Besides these animals, other animals did survive and it should be taken into consideration that these survivors are not included in this studie. The exact number of succesfully rehabilitated seals is unknown. So we can only speculate about the succesrate of Ecomare and the succes of the lungworm treatment that was used.
Because of the great amount of lungworm patients and the intensive treatment needed, the employees of Ecomare wondered wether it was possible to make a diagnosis on the severity of the lungworm infection, based on external observations and symptoms. When possible, the desicion between starting the treatment or euthanasia could be taken at a seals arrival. Unfortunately this question could not be answerd based on the results of this studie due to freezing artefacts, the given treatment and abscence of information about the clinical symptoms. It was impossible to find a correlation between the severity of the infection, damage to the lungtissue and the clinical symptoms at arrival.
More research should be done to create a clear policy on when to start treatment and when to euthanise a lungworm patient arriving at Ecomare. In a follow up study different factors should be taken into consideration.
- First only fresh animals (DCC 1) should be used for post mortem examination. Freezing causes major artefacts on the lungtissue. Microscopical evaluation will be much more usefull when only DCC 1 carcasses are being used.
- Secondly more documentation on background, treatment and symptoms will be needed. Symptoms should be documented according to a clear classification or score system. This way, data can be used to apply statistical analysis.
- Finally, it could prove usefull to perform x-rays and CT-scans to obtain more information on the damage of the lungtissue whilst clinical research is limited.

Further research

When more research would be done on this subject multiple things will have to be improved. First of all, more time will be needed to perform more necropsies including microscopical evaluations over a set period of time. For example, all the stranded seals in on year or the fall and winter season.
Secondly, animals arriving at Ecomare should be examined and a possible diagnosis should be made. When this animal survives and would be released this data can be included in the studie and the population becomes more representive.
Finally a detailled classification system should be developed to objectively categorize the severity of a lungworm infection. This should include the amount of damage but also the number of parasites.

Conclusion

In 27 stranded seals (November 2011 – January 2012) different causes of death like trauma, have been found but appeared to be not as significant as lungworm infections.
From the results that have been found, and the results of earlier studies, the conclusion can be drawn that the lung parasites Otostrongylus circumlitus and Parafilaroides gymnurus must be regarded as an important cause of natural mortality for the seals of the Dutch Waddensea[11]. Multiple other helminth species were found but not designated as the cause of death.
More research is needed to find a correlation between the severity of the lungworm infection, damage to the lungtissue and the clinical symptoms when stranded. This would be of great value for rehabilitation centres like Ecomare.

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Attachments

Dissection protocol

Part 1 Identification	Number	…….....	GLIMS	.....
	Stranding date:			
	Autopsy date:			
	Autopsied by:			
Chip check[1]:				
□ yes / □ no	True location:	.....	NSO	.....
negative / positive	Provided by:	□ EHBZ □ EcoMare □ Other		

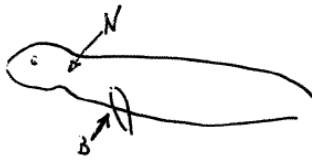

Diagram 1 – blubber thickness (including skin)

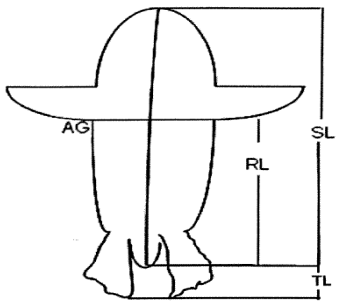

Diagram 2 - morphometry

Part 2 Biometrics	Morphometry (see diagrams above)	Blubber thickness **neck** (N)..... mm Blubber thickness **breast** (B)..... mm	**TL**.....cm **SL**.....cm **RL**.....cm **AG** (axillary girth).....cm
Sex:	□ ♂ □ ♀ (certain / uncertain) □ sex unknown		♂ large anogenital distance ♀ vulva located just ventral to anus
Body mass:	.....kg		real / estimation
Nutritive condition code:	□ NCC**1** □ NCC**2** □ NCC**3** □ NCC**4** □ NCC**5** □ NCC**6**		
Storage:	□ Direct delivery □ Cooled (ca. ……hrs) □ frozen □ other		

Expected age:	□ Neonate □ Juvenile □ Adult □ Unknown
Decomposition DCC:	□ Very fresh DCC**1** □ Fresh DCC**2** □ Putrefied DCC**3** □ Very putrefied DCC**4** □ remains DCC**5**
State of carcass:	□ fully intact □ peck or bite wounds □ incomplete □ skeletal parts, namely: ……..……
Bycatch: (based on external observation only)	□ certain □ highly probable □ probable □ possible □ no evidence
Part 2 Photography	
Entire body	
Head only	
Snout	
Eyes	
Teeth	
Urogenital region	
External Observations (Specify lesion and location)	
Internal observations (Specify organ)	

Estimated significance of the presence/absence of criteria for the diagnosis of bycatch			
Criteria	**Presence**	**Absence**	**Observed**
1. Health state			**yes ? no**
A. Exclusion of other causes of death	+	--	□ □ □
B. Good nutritional condition	+	-	□ □ □
C. Evidence of recent feeding	+	0	□ □ □
2. Contact with fishing gear			
A. Superficial skin lesions			**yes ? no**
1. cuts in edge of mouth, fin or tail	++	0	□ □ □
2. encircling lesions around extremity	++	0	□ □ □
B. Bruises	+	0	□ □ □
C. Skull fractures	+	0	□ □ □
3. Lack of oxygen (hypoxia)			**yes ? no**
A. Oedematous lungs	+	-	□ □ □
B. Persistent froth in the airways	+	-	□ □ □
C. Bullous emphysema in the lungs	+	0	□ □ □
D. Epicardial and pleural petechiae	+	0	□ □ □
4. Damage during release of the net			**yes ? no**
A. Amputated fin, fluke or tail	++	0	□ □ □
B. Penetrating incision into body cavity	++	0	□ □ □
C. Rope around tail stock	++	0	□ □ □
D. Gaff mark	++	0	□ □ □
5. Other relevant characteristics			**yes ? no**
A. Sharp edged cuts or blubber defects on body	++	0	□ □ □
B. Sharp edged cuts or blubber defects on mandible	++	0	□ □ □
			□ □ □

++ consistent with bycatch + bycatch possible 0 no significance for diagnosis - bycatch less likely -- bycatch unlikely

[1]Kuiken T. 1994. Review of the criteria for the diagnosis of by-catch in cetaceans. *In:* Kuiken T. (ed.) Diagnosis of By-Catch in Cetaceans. Proc. 2nd. ECS workshop on cetacean pathology, Montpellier, France, 2 March 1994. European Cetacean Society Newsletter 26: 38-43

Part 3 Pathology	Number	.....	GLIMS	.....
Necropsy form – 1				
External observations & lesions				
□ Scavenging	□ Severe	□ Moderate	□ Mild	□ None

Subcutaneous observations & lesions □ Subcut. fat	□ Absent □ Present, approximate thickness

Part 3 Pathology	Number	.....	GLIMS	.....
Necropsy form - 2				

Internal observations & lesions

Abdomen (tick if normal, describe if abnormal) □ Urinary bladder □ Mesenteric LN □ Intestine □ Stomach □ Spleen □ Pancreas □ Liver □ Adrenal □ Kidney □ Genital tract □ Gonads	Sex □ ♂ □ ♀ □ ND Age □ Neonatal □ Juvenile □ Adult □ Undetermined
Thorax (tick if normal,	

describe if abnormal) □ Trachea □ Lungs □ Bronchial LN □ Heart □ Oesophagus □ Thymus (present/absent)	

Part 3 Pathology	Number	.....	GLIMS	.....
Necropsy form - 3				

Head and Neck (tick if normal, describe if abnormal) □ Larynx □ Thyroid □ Oral cavity □ Nostrils □ Eyes □ Teeth □ Auditory system □ Skull □ Brain	
Conclusions	

Probable cause of death	

Part 6 Sample Collection	Number	.....	GLIMS	.....
Sample list				

Pulmonary LN				Alchol / Glycerin	**TX Alu**	**TX PL**	**TX PL**	**Life History**
Heart			Lesions					Skin&Hair
Skin								
Blood & / Serum					3x TX	TX	TX	
Blubber								
Thymus					TX	TX	TX	
Muscle								
Thyroid			Swab					
Genital split								
Eye								
Mam.gland/penis								2x Mandible
Teeth								
Gonad & reproductive								
Cerebellum								
tract								
Cerebrum								
Reproductive tract LN								
Intestine								
Placenta, umbilical cord								
Intestinal contents								
Urinary bladder								Ontkalking
Rib cartilage								
Ileocecale LN								
Mesenteric LN								
Pre scapular LN								
				Parasites			SB DCC 4 and 5	
Stomach	DCC 1		DCC 2		DCC 3			
Collection/ DCC								
Pancreas								
correlation								
Spleen								
Liver				Parasites	3x TX	TX	TX	
Kidney					3x TX	TX	TX	
Adrenal								
Lung			Parasites	Parasites				

Macroscopy template

Macroscopy	Number		GLIMS	
	Autopsy date			
DCC		Age	Sex	NCC	
External Observations & lesions					
General Observations					
Skin Lesions					
Net marks					
Cuts in mouth					
Cuts on fins					
Cuts on fluke					
Other cuts					
Blubber defects					
Encirc. Lesions					
Penetrating incisions					
Amputations					
Rope marks					
Gaff marks					
Others					
Scavenging					

Subcutaneous Observations & lesions	
Blubber	
Subcutis	
Musculature	
Skeleton	
Mammary Gland	
Subcut fat	
Internal Observations	
Abdomen	
Urinary Bladder	
Mesenteric LN	
Intestine	
Stomach	
Spleen	
Pancreas	
Liver	
Adrenal	
Kidneys	
Genital Tract	

Genital Tract LN	
Gonads	
Thorax	
Trachea	
Lungs	
Pulmonary LN	
Heart	
Oesophagus	
Thymus	
Head and Neck	
Nose	
Larynx	
Thyroid	
Oral Cavity	
Teeth	
Eyes	
Auditory system	
Skull	
Brain	

Preliminary Conclusions	
Probable cause of death	
Voorlopig resultaat voor de vinder (NL)	

Further comments:

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